AD-A087 931 AIR FORCE AEROSPACE MEDICAL RESEARCH LAB WRIGHT-PATT--ETC F/G 14/2
A TECHNIQUE FOR THE INJECTION OF RADIOACTIVE TRACER MICROSPHERE--ETC(U)
JUN 80 K J GREENLESS, C M OLOFF, W J BUEHRING

UNCLASSIFIED AMRL-TR-78-114 NL

1 OF 1
AD A087931

END
DATE FILMED
9-80
DTIC

AMRL-TR-79-114

LEVEL II

A TECHNIQUE FOR THE INJECTION OF RADIOACTIVE TRACER MICROSPHERES DURING ACCELERATION STRESS

KEVIN J. GREENLEES
CLARENCE M. OLOFF
WILLI J. BUEHRING
KATHERINE C. SMITH

JUNE 1980

AIR FORCE AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433

NOTICES

When the Government [illegible] specifications, or other data are used for any purpose other than a definitely related Government [illegible] Government thereby incurs no responsibility nor any obligation whatsoever, and the [illegible] formulated, furnished, or in any way supplied the said drawings, specifications, or [illegible] licensed by implication or otherwise, as in any manner licensing the holder or any other [illegible] conveying any rights or permission to manufacture, use, or sell any patented invention that [illegible] related thereto.

Please do not request copies of this report from Air Force Aerospace Medical Research Laboratory. Additional copies may be purchased from:

A TECHNIQUE FOR THE INJECTION OF
RADIOACTIVE TRACER MICROSPHERES
DURING ACCELERATION STRESS

[illegible]

Federal Government agencies and their contractors registered with Defense Documentation Center should direct requests for copies of this report to:

Defense Documentation Center
Cameron Station
Alexandria, Virginia 22314

KENNY GREENLEES
CLARENCE M. CLOFF
WILLY BOEHRING
KATHERINE C. SMITH

TECHNICAL REVIEW AND APPROVAL

AMRL-TR-78-114

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

JUNE 1980

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Henning E. von Gierke

HENNING E. VON GIERKE
Director
Biodynamics and Bioengineering Division
Air Force Aerospace Medical Research Laboratory

AIR FORCE/86780/29 June 1980 — 150

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
AMRL-TR-78-114	AD-A087 937	

4. TITLE (and Subtitle)

A TECHNIQUE FOR THE INJECTION OF RADIOACTIVE TRACER MICROSPHERES DURING ACCELERATION STRESS,

5. TYPE OF REPORT & PERIOD COVERED

Technical Report

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)

Kevin J. Greenlees Katherine C. Smith
Clarence M. Oloff
Willi J. Buehring

8. CONTRACT OR GRANT NUMBER(s)

9. PERFORMING ORGANIZATION NAME AND ADDRESS

Aerospace Medical Research Laboratory, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson Air Force Base, Ohio 45433

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS

62202F 7222-10-50
6893-07-02

11. CONTROLLING OFFICE NAME AND ADDRESS

12. REPORT DATE

June 1980

13. NUMBER OF PAGES

17

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)

15. SECURITY CLASS. (of this report)

UNCLASSIFIED

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

Supported in part by the Laboratory Director's Fund.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Microspheres
Blood Flow
Acceleration

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A microsphere injection system is presented which allows the remote sequential injection of as many as five seperately labeled radioactive tracer microspheres while under acceleration stress. Cinemicroscopic observation of the injected microsphere suspension showed little or no microsphere aggregation. Statistical analysis of the microsphere injections on the basis of counts per minute showed 90 + 5.7% of the injection was delivered during three gravities of acceleration. No significant difference in percent injectate delivered was found among the five cartridges. Subjection of the microsphere injection system to 12 G acceleration

over

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

20 continued.

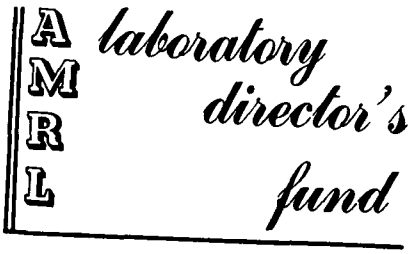
did not impair proper sequencing or delivery of test solutions.

PREFACE

The work presented here was conducted by personnel of the Aerospace Medical Research Laboratory, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson Air Force Base, Ohio, under Project 68930702.

We wish to express our thanks to Mrs. Sharon Ward for her many helpful suggestions during the evaluation of the techniques.

This research was supported in part by the Laboratory Director's Fund.



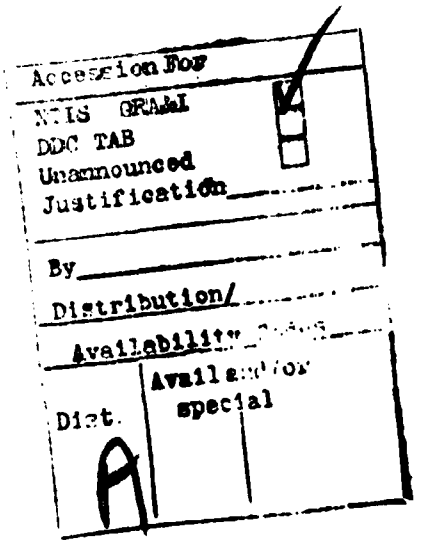


TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

INTRODUCTION

Classically, the experimental use of radioactive tracer microspheres to study blood flow (Rudolph and Heymann, 1967), specifically cerebral blood flow (Marcus et al., 1976), has taken place in the laboratory under the immediate control of the investigator. The injection of radioactive microspheres under dynamic conditions, such as acceleration stress studies, involves a number of technical difficulties. Preparation of the microsphere suspensions is preferably done in a properly equipped and supervised radiobiological laboratory. Frequently, however, the experimental subject is to be stressed at another location. The investigator is thus forced to handle the radioactive tracer microspheres under less than ideal conditions. In past acceleration stress studies only a single prepared injection could be remotely injected while the animal was stressed (Hamlin and Leverett, 1976; Soetre et al., 1977).

Research on the effects of high acceleration on blood flow distribution in the brain using subhuman primates necessitated an investigation of the problem of multiple injections in a dynamic environment. We were concerned with the injection of radioactive tracer microspheres (3M Tracer Microspheres, 3M Co., Minnesota) at specific points during the acceleration profile. The system presented here allows the remote sequential injection of as many as five separately labeled microspheres during acceleration stress. The separate manifold assembly permits loading the radioactive tracer microspheres in a laboratory equipped to handle radioisotopes safely. The loaded manifold assembly can then be transported as a unit to the site of experimentation. This greatly reduces the risk of radioactive spills and minimizes delay during the experiment. Use of common laboratory materials at many points allows easy replacement of contaminated or damaged parts. Isolation of the microsphere suspensions and an effective rinse permits the repeated use of the manifold assembly.

MATERIALS METHODS

The microsphere injection system (patent pending) is composed of six basic parts; the first five of which are illustrated in Fig. 1:

1. The reservoir system consists of a 100 ml stainless steel pail suspended between two supporting arms. A gimbal arrangement [2] leaves the pail free to swivel at the point of contace. The two supporting arms are fastened to the edge of the platform supporting the test animal. A 250-ml or 500-ml injection bottle [3] placed in the pail serves as a reservoir for saline. The pail movement under acceleration maintains the injection bottle in proper alignment with the G-force vector. A disposable needle [4] in the stopper of the injection bottle serves as an air vent.

2. A laboratory fabricated "T" [5] constructed from a modified three-way stopcock is connectea to the reservoir system by polyethylene tubing. The tubing is forced through the injection bottle stopper to the bottom of the bottle. A one-way valve in line at the "T" prevents flow back to the reservoir system. From the common joint in the "T" one line of tubing goes to the microsphere sequencer [11] and another to a 20-ml nylon luer-lock syringe [7] on the Sage® pump [6].

3. A standard Sage pump [6] is used, unmodified except for a clamp holding the "T" [5] firmly to the pump (model 197, SAGE Instrument Inc., NY). A three-way stopcock [8] between the nylon syringe [7] and the polyethylene tubing permits venting of air from the syringe and lines. The pump is adjusted to deliver 15 ml with each injection.

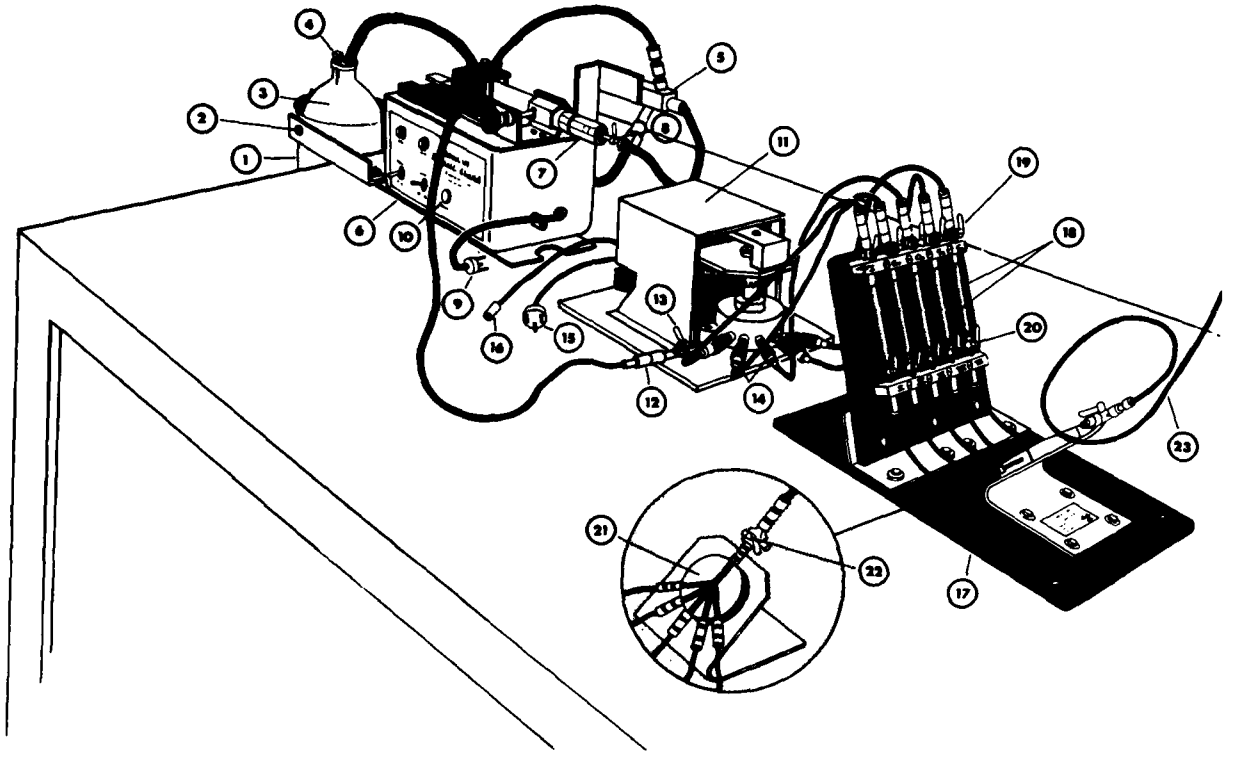
4. The microsphere sequencer [11] is attached to the tubing from the "T" [5] by a one-way valve [12] and a three-way stopcock [13] in series. The one-way valve prohibits flow back from the sequencer. The stopcock between the one-way valve and the sequencer permits venting of air from the lines. The microsphere sequencer is a laboratory fabricated device. A gear drive electrically rotates to permit sequential flow through each of a series of five channels. A one-way valve [14] at each of these channels prevents backflow from the microsphere sequencer. Polyethylene tubing from each valve connects to a separate cartridge [18] on the manifold assembly [17] for each channel in the microsphere sequencer [11].

5. The manifold assembly [17] is laboratory fabricated. The base is made of bakelite, with nonporous easily cleaned surfaces. To the bottom board (35 mm x 20 mm) a second board (18 mm x 17 mm) is attached at a 70 degree angle. Clamped to this upright section are five modified one-ml disposable plastic syringe bodies [18] that serve as cartridges. Tubing from each discrete channel in the microsphere sequencer is attached to the corresponding cartridge by a three-way stopcock. [19] Another two-way stopcock [20] at the base of each cartridge serves to isolate the cartridge from the rest of the system as required. Polyethylene tubing from each cartridge connects to a discrete channel in a plexiglass manifold [21]. The channels are bored in such a manner that each joins to a common exit channel. This common channel opens to a three-way stopcock [22]. This is the point of attachment for the catheter [23] placed in the test animal. The manifold [21] is mounted on the base at a 45 degree angle. When loaded, the microspheres rest in the polyethylene tubing between the manifold and the cartridges [18]. They are efficiently rinsed from the system by each subsequent injection.

6. The final part of the system, the microsphere loading device (patent pending), is illustrated in Fig. 2. It is constructed of two modified three-way valves [1] in series and a modified 19-gauge stainless steel needle [2]. The device allows the one-ml loading syringe [3] used to load the microspheres to be efficiently rinsed. A 5-ml syringe [4], used as a saline reservoir, allows fresh saline to be drawn into the one-ml syringe [3] and flushed into the cartridge of the manifold without loss of microspheres or contamination of the saline reservoir. Grooves in the 19-gauge needle [5] allow air to escape and prevent air bubbles.

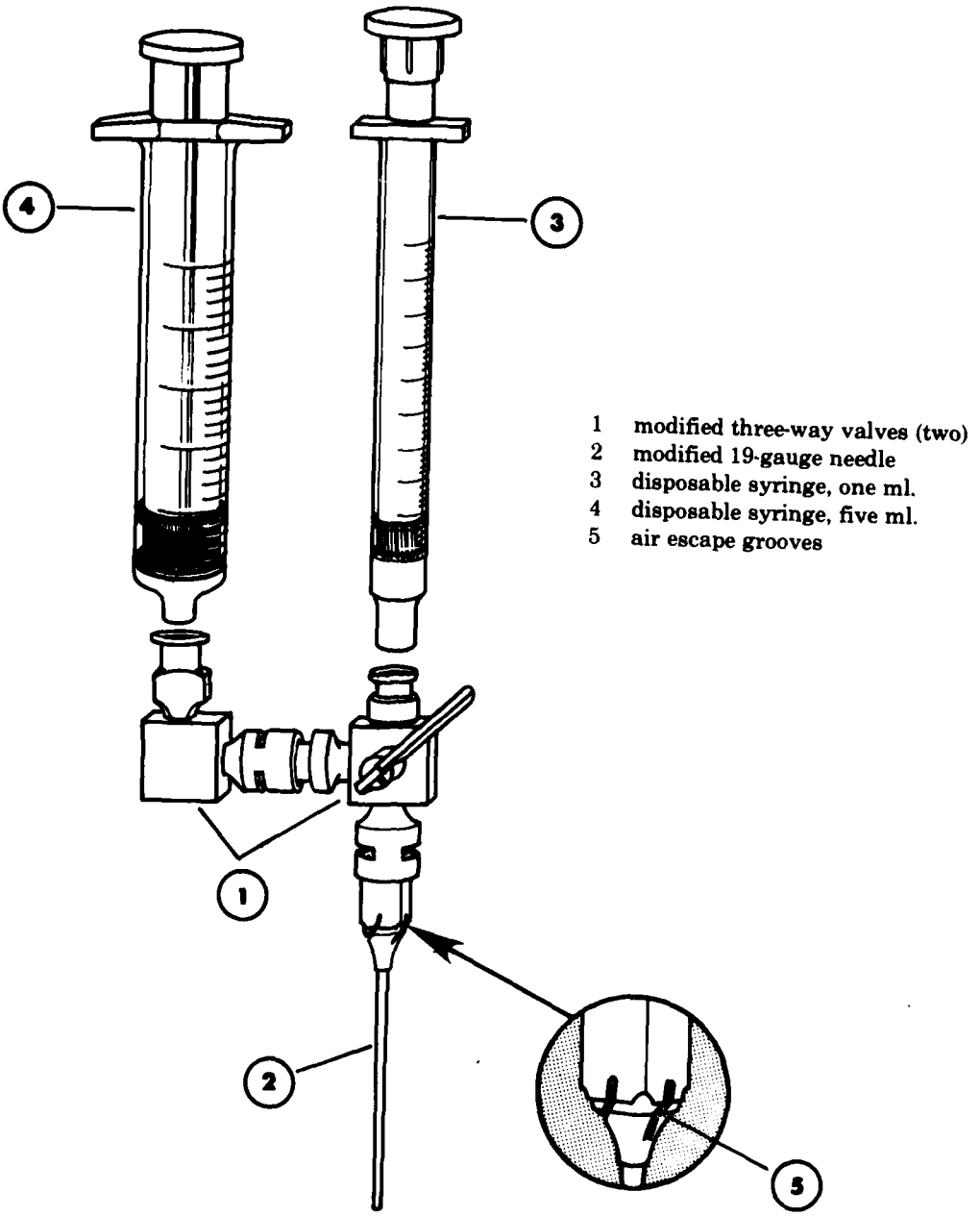
The manifold assembly, as illustrated in Fig. 3, should be loaded with the microsphere suspensions in a laboratory equipped to handle radioisotopes safely.

A suspension of microspheres in 0.005% Tween-80 (Fisher Scientific Co., Pittsburgh) and 5% dextran was prepared one day in advance. Each cartridge of the manifold assembly was loaded at 30-minute intervals



1 stainless steel pail
2 gimbal
3 injection bottle
4 disposable needle
5 "T"
6 sage pump
7 nylon syringe, 20 ml.
8 three-way stopcock
9 AC-power cable
10 remote control outlet
11 microsphere sequencer
12 one-way valve
13 three-way stopcock
14 one-way valves (five)
15 AC-power cable
16 remote control cable
17 manifold assembly
18 cartridges (five)
19 three-way stopcocks (five)
20 two-way stopcocks (five)
21 plexiglass manifold
22 three way stopcock
23 catheter to subject

Figure 1. Microsphere Injection System



1 modified three-way valves (two)
2 modified 19-gauge needle
3 disposable syringe, one ml.
4 disposable syringe, five ml.
5 air escape grooves

Figure 2. Microsphere Loading Device

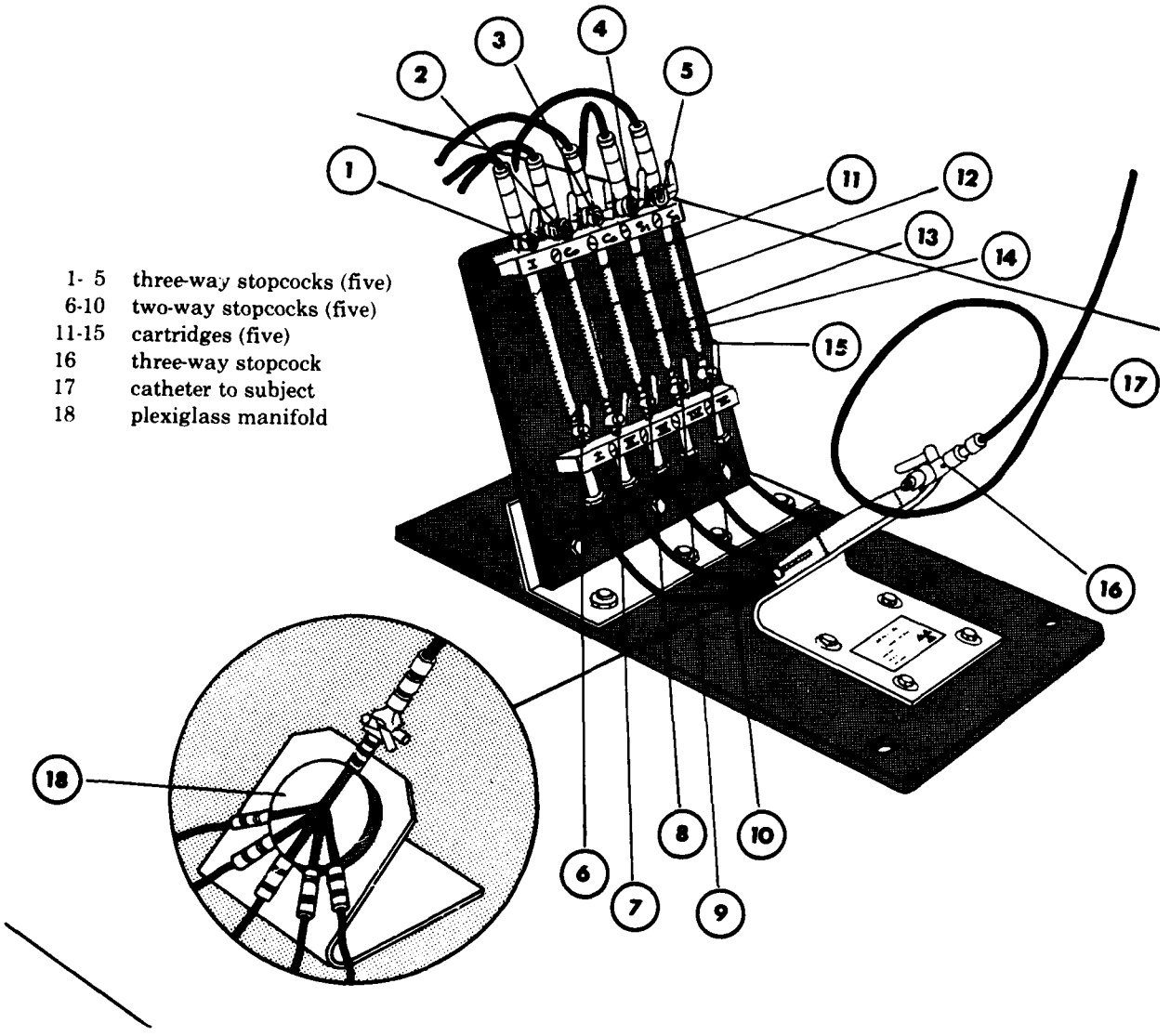
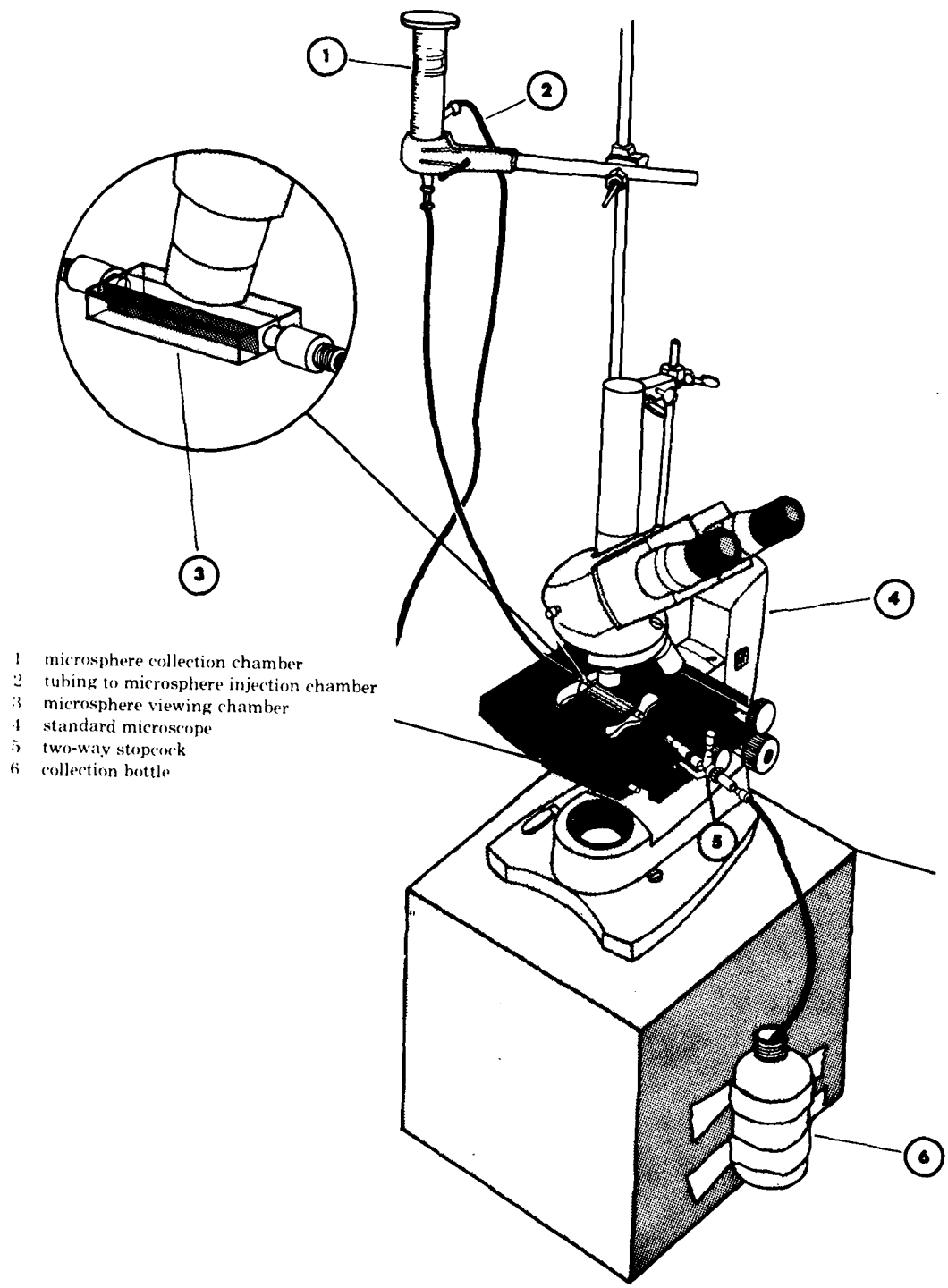


Figure 3. Manifold Assembly



1 microsphere collection chamber
2 tubing to microsphere injection chamber
3 microsphere viewing chamber
4 standard microscope
5 two-way stopcock
6 collection bottle

Figure 4. Microsphere Viewing and Collection Chambers

following thorough mixing of the microspheres suspension on a Vortex® mixer. Each cartridge received approximately one million microspheres in 0.5 ml of Tween-80-5% dextran solution. The microsphere injectant from each cartridge was viewed separately. Refer to Fig. 4 in the procedure outlined. A laboratory fabricated microsphere collection chamber [1] received the microsphere injection, mixing the microspheres into the 15 ml of saline flushed through the microsphere injection system. From this point the microsphere suspension was gravity fed through polyethylene tubing, past a specially constructed microsphere viewing chamber [8]. A standard stopcock [5] was used to control the flow rate. The microspheres were observed under a microscope [4] at low power (100X). High speed cinemicroscopy was used to observe the microspheres at faster flow rates, using a magnification of 10X and a speed of 400 frames per second.

In conjunction with an ongoing study of cerebral blood flow (Yoder et al., 1978), the microsphere injection system was mounted on the animal platform of the Dynamic Environment Simulator (DES); a closed-loop man-rated centrifuge in the Air Force Aerospace Medical Research Laboratory. The injection system was connected to a conscious baboon confined in the Oloff Primate Restraint System (patent 4120266) placed on the DES animal platform.

The cartridges were loaded with 0.2 ml to 0.8 ml of the following microsphere suspensions: Iodine-125, Cerium-141, Chromium-51, Strontium-85 and Scandium-46. The first microsphere suspension was injected under static 1 Gz conditions. The remaining four suspensions were sequentially injected at an acceleration of 3 Gz. This was repeated on four separate occasions. Statistical analysis was performed on the basis of counts per minute (CPM) to determine the reliability of injection from cartridge to cartridge and the repeatability of injections for the entire system.

The percent injected for each experiment was determined by the following equation:

$$\% \text{ injected} = \frac{\text{count of loading syringe} \times \text{count of residue in loading syringe} \times \text{rinse of manifold assembly}}{\text{count of loading syringe} \times 1/100}$$

Cartridge to cartridge repeatability was determined by an analysis of variance.

Finally, the microsphere injection system alone was subjected to 12 Gz acceleration stress on the DES. All samples of microsphere suspensions were counted in a Packard Auto-Gama Spectrometer®.

RESULTS

High speed cinemicroscopy of the microsphere suspension showed very little aggregation of the microspheres (fig. 5) until the microspheres had been allowed to remain in the manifold assembly for more than 2 hours (fig. 6). Subjection of the microsphere injection system to 12 Gz acceleration did not impair proper sequencing or delivery of nonradioactive microspheres and did not impair proper sequencing or delivery of nonradioactive test solutions.

Statistical analysis of the microsphere suspension injections at 3 Gz showed that 90% + 5.7% of the suspension was injected each time. No significant difference was found among cartridges in percent delivered. The percentage of the microsphere suspension injected decreased as the volume of microsphere suspension increased. The best percentage was obtained from Iodine-125 which had a volume of 0.2 ml, with 97.9% of the loaded suspension being injected.

DISCUSSION

It is important for any microsphere injection technique to deliver a well mixed microsphere suspension (Buckberg et al., 1971). This means that there is little or no microsphere aggregation. The microspheres are meant to mimic the distribution of erythrocytes in the blood. They are selected by size so that they are trapped in specific capillary beds. For our test purposes we chose 15 micrometer microspheres. This is considered the size best suited for studies of cerebral flow (Marcus et al., 1976). Aggregation of the microspheres, as illustrated in Fig. 6, would adversely alter the distribution of the microspheres in the vascular system. The accepted method to prevent this is to use a surface active agent such as Tween-80.

Millard et al. (1977), have found that injection of Tween-80 can cause adverse reactions on blood pressure, heart rate, and cardiac dimensions. They recommend a maximum concentration of 0.01% Tween-80 to prevent the cardiovascular effects. Our studies showed 0.005% Tween-80 in 5% dextran to be adequate to prevent aggregation with the volumes used. The microsphere suspensions were injected into a reservoir and drained slowly over a 30-60 second period for cinemicroscopy. This slow rate provides adequate opportunity for aggregation. Injection into the ventricle through a catheter, such as that used for an animal, typically requires less than 10 seconds. The actual time depends on the catheter length and diameter. Such faster flow rates provide less opportunity for aggregation.

The statistical results indicate that the microsphere injection system reliably delivers the microsphere suspensions. The percentage of suspensions actually injected improves as the volume of suspension is decreased. This smaller volume, in the 0.2- to 0.4-ml range, permits a more efficient rinse of the cartridge during injection. The counts per minute of the microsphere suspension actually injected may easily be determined by subtracting the residue in the loading syringe and the counts from a rinse of the manifold assembly from the actual counts in the loading syringe before filling the cartridge

The microsphere injection system is an effective tool for the injection of radioactive tracer microspheres. The isolation of the microspheres in the manifold assembly and the extensive use of one-way valves, prevent contamination of the otherwise clean system by the radioactive suspensions. Disposable components in the manifold assembly prevent cross-contamination between experiments. These facts and the ability to limit all handling of the microspheres to a properly equipped laboratory, decrease the potential hazard inherent when using radioactive materials.

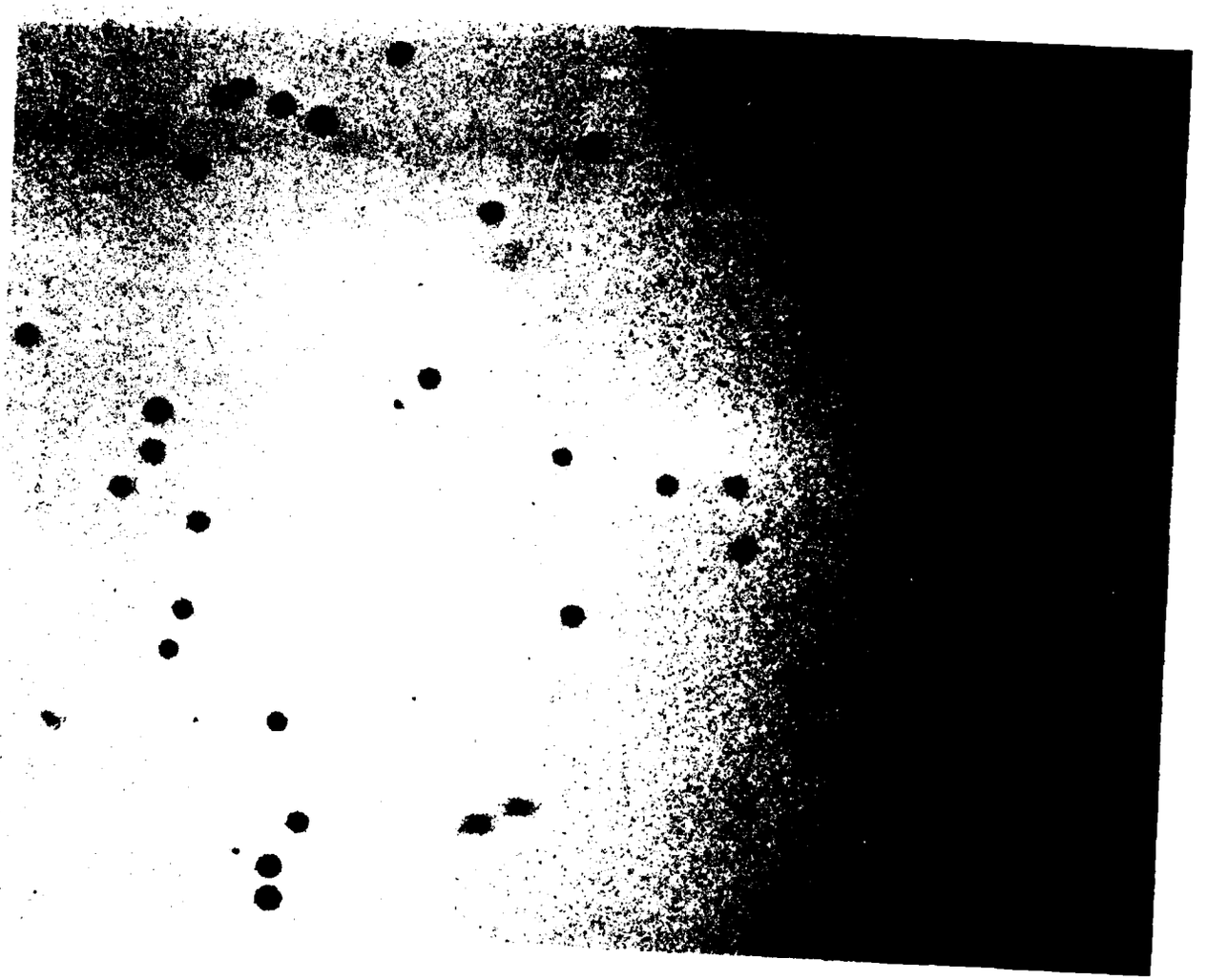


Figure 5. Non-Aggregated Microspheres in Suspension

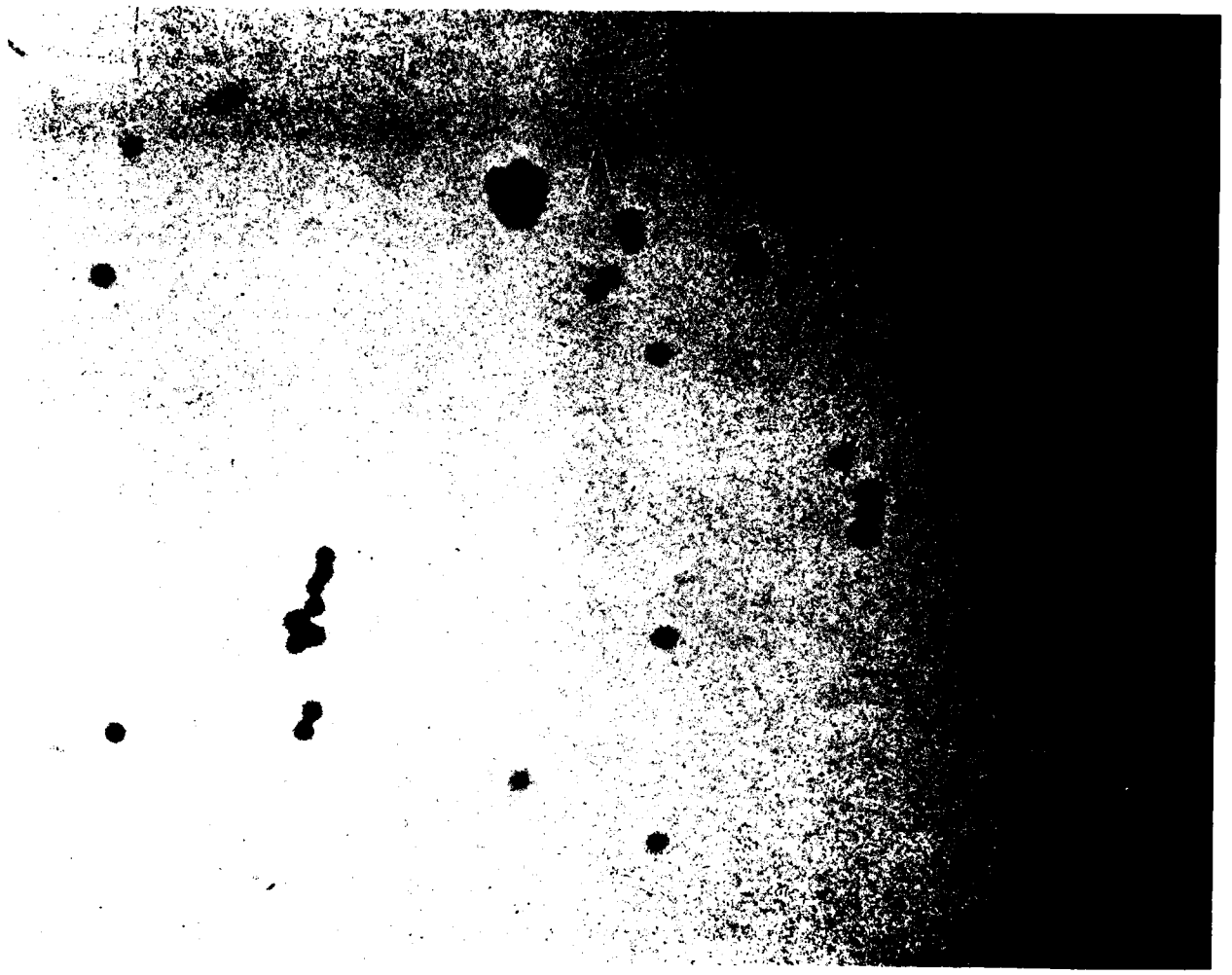


Figure 6. Aggregated Microspheres in Suspension

REFERENCES

1. Buckberg, G.D., J.C. Luck, D.B. Payne, J.L.E. Hoffman, J.P. Archie, and D.E. Fixler (1971), "Some Sources of Error in Measuring Regional Blood Flow with Radioactive Microspheres," *J. of Appl. Physiol.* 31 (4), 598-604.

2. Hamlin, R.L., and S.D. Leverett (1976), "Effects of Sustained +Gz Acceleration on the Cardiac Output and Fractionation of Cardiac Output in Awake Miniature Swine, *AGARD Conference Proceedings*, No. 189.

3. Marcus, M.L., D.D. Heistad, J.C. Ehrhardt, and F.M. Abboud (1976), "Total and Regional Cerebral Blood Flow Measurement with 7-, 10-, 15-, 25-, and 50-Micrometer Microspheres," *J. of Appl. Physiol.* 40 (4), 501-507.

4. Millard, H. Baig, and S. Vatner (1977), "Cardiovascular Effects of Radioactive Microsphere Suspensions and Tween-80 Solutions," *Am. J. of Physiol.* 232 (3), H331-H334.

5. Rudolph, A.M., and M.A. Heymann (1967), "The Circulation of the Fetus in Utero: Methods for Studying Distribution of Blood Flow, Cardiac Output and Organ Blood Flow," *Circulation Res.* 21, 163-184.

6. Sostre, S.J., A. Kennealy, J.S. Kirkland, C.M. Oloff, A.A. Karl, and M. Franey (1977), "Cerebral Blood Flow in Baboons Under Positive Acceleration," Aerospace Medical Association, Annual Scientific Meetings, 75-76.

7. Yoder, J.E., A.A. Karl, C.M. Oloff, and K.J. Greenlees (1978), "A Comparison of Invasive Techniques for Assessment of Cardiac Output Under Acceleration Stress," Aerospace Medical Association, Annual Scientific Meetings.

*U.S.Government Printing Office: 1980 — 657-084/766